Distribution of Spotted Gar (*Lepisosteus oculatus*) adults and juveniles in the Rondeau Bay, Long Point Bay, and Hamilton Harbour watersheds

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ABSTRACT

The Spotted Gar, a Threatened species in Canada, is found in warm, shallow waters of several Great Lakes coastal wetlands. Despite recent research, information on the distribution and habitat use of this species, particularly the juvenile life stage, is limited. This study combines traditional sampling techniques and environmental DNA (eDNA) sampling to determine the occurrence of Spotted Gar populations in Hamilton Harbour and Cootes Paradise Marsh in Lake Ontario, as well as the potential use of tributary streams for spawning in Rondeau Bay. Juvenile Spotted Gar habitat use was also examined in Long Point Bay, Rondeau Bay, and its adjacent tributaries. No Spotted Gar were found in Hamilton Harbour, although a positive eDNA detection raises the possibility of a population in the adjacent Cootes Paradise. Using traditional sampling methods in Cootes Paradise resulted in no Spotted Gar captures. Spotted Gar were found to be utilizing the tributaries in the Rondeau Bay watershed, indicating that it occurs in tributaries extending from the bay to the first permanent barrier to fish passage. Juvenile Spotted Gar were captured using seine nets and were found to inhabit shallow (<0.5 m) waters with abundant aquatic vegetation. Sampling in Long Point Bay resulted in no captures of juvenile Spotted Gar, indicating low levels of successful reproduction, thus, the long-term viability of the Long Point Bay population of Spotted Gar is uncertain.

RÉSUMÉ

Le lépisosté tacheté est une espèce menacée du Canada que l'on trouve dans les eaux chaudes et peu profondes de plusieurs zones humides côtières des Grands Lacs. Bien que des recherches aient été menées sur le sujet récemment, on dispose de peu de renseignements sur la répartition et l'utilisation de l'habitat de cette espèce, particulièrement en ce qui concerne les juvéniles. La présente étude combine les techniques classiques d'échantillonnage et l'échantillonnage de l'ADN environnemental afin de déterminer la présence de populations de lépisosté tacheté au port de Hamilton et dans le marais Cootes Paradise, dans le lac Ontario, ainsi que l'utilisation potentielle des affluents pour frayer dans la baie Rondeau. L'on s'est également penché sur l'utilisation de l'habitat par les lépisostés tachetés juvéniles dans la baie Long Point, dans la baie Rondeau et dans les affluents qui sont adjacents à celle-ci. Aucun lépisosté tacheté n'a été trouvé au port de Hamilton, mais l'échantillonnage de l'ADN environnemental a permis de détecter la présence potentielle d'une population de cette espèce dans le marais adjacent Cootes Paradise. L'utilisation de techniques classiques d'échantillonnage à Cootes Paradise n'a pas permis de capturer des lépisostés tachetés. L'on a découvert que le lépisosté tacheté utilise les affluents du bassin versant de la baie Rondeau, ce qui indique la présence de l'espèce dans les affluents qui s'étendent de la baie jusqu'au premier obstacle permanent au passage du poisson. Des lépisostés tachetés juvéniles ont été capturés à la senne, ce qui indique que ce poisson vit dans les eaux peu profondes (moins de 0,5 m) qui renferment une végétation aquatique abondante. L'échantillonnage effectué dans la baie Long Point n'a pas permis de capturer des lépisostés tachetés juvéniles, ce qui indique un niveau peu élevé de reproduction réussie. La viabilité à long terme de la population de lépisosté tacheté de la baie Long Point est donc incertaine.

INTRODUCTION

The Spotted Gar (*Lepisosteus oculatus*), a large predatory fish, is listed as a Threatened species in Canada under the federal *Species at Risk Act*, and in Ontario under the provincial *Endangered Species Act*. In Canada, this species is at the northern edge of its range and populations are found in three coastal wetlands of Lake Erie (Long Point Bay, Rondeau Bay, Point Pelee; COSEWIC 2005). This limited distribution and potential for habitat loss is the reason for the designation of this species as Threatened (COSEWIC 2005). Outside of the three known populations, single specimens have been verified in Muddy Creek of the Lake Erie watershed (DFO, unpublished data) as well as East Lake and Hamilton Harbour in Lake Ontario (Staton et al. 2012). Additionally, historical records show single individuals have been collected from Lake St. Clair and Bay of Quinte (Staton et al. 2012). To date, the existence of populations in these areas where single records have originated is yet to be verified.

Spotted Gar live in warm, shallow habitats (Scott and Crossman 1998; Snedden et al. 1999; Glass et al. 2012) with plentiful structure, which is used for cover and to ambush prey. Radio-tracking surveys of Spotted Gar populations have shown that woody debris is a favoured form of cover in Louisiana (Snedden et al. 1999), while the Rondeau Bay population favours aquatic vegetation (Glass et al. 2012). Although populations in varying habitats can be found in different types of cover, the complexity of three-dimensional cover is important and affects the feeding success of the species (Ostrand et al. 2004). Spotted Gar begin to move into shallow waters near shore as water temperatures approach 15°C (Glass et al. 2012). Spawning takes place in the spring in shallow water, where adhesive eggs are laid among vegetation (Redmond 1964). Newly hatched Spotted Gar remain attached to vegetation using a suctorial disk on the dorsal side of the snout, present in individuals smaller than 17.6 mm (Simon and Wallus 1989). Once the yolk sac is absorbed, the adhesive organ also disappears and the larvae are free swimming (Simon and Wallus 1989). Spotted Gar in Canada become sexually mature at three years of age (Glass et al. 2011).

Despite recent studies on the Rondeau Bay population of Spotted Gar, this remains a relatively understudied species in Canada. One of the key knowledge gaps is the habitat use by the juvenile life stage (Bouvier and Mandrak 2010). Mortality at the juvenile life stage was predicted to have the largest effect on the long-term recovery of the species in Canada (Young and Koops 2010), thus, knowledge of the habitat utilized by the juvenile life stage is of vital conservation importance. The objective of this study is to answer several of the key knowledge gaps regarding this species in Canada. The objectives are to determine: the occurrence of a population in Hamilton Harbour and Cootes Paradise; the use of tributaries by the Rondeau Bay population; and, habitat use by the juvenile life stage in Rondeau Bay and Long Point Bay. The knowledge gained about occurrence of the species in Hamilton Harbour and Cootes Paradise, as well as the use of Rondeau Bay tributaries, will be important for protecting critical habitat of the species in Canada. Spawning has been documented in Rondeau Bay (Glass et al. 2012) but, to date, has not been observed in the tributaries. The tributaries of Rondeau Bay are agricultural drains, potentially subject to agricultural drain maintenance that would remove vegetation and increase flow and turbidity, negatively impacting the suitability of the habitat for this species.

METHODS

HAMILTON HARBOUR FYKE NETTING

Fine-mesh fyke nets (1.2 m diameter, 6.35 mm mesh) were set at 14 locations with suitable shallow water habitat in Hamilton Harbour (Figure 1) between June 2, 2011 and June 14, 2011, for a total of 19 discrete samples. Nets were set at shallow water locations to target potential spawning aggregations and were in place for approximately 24 hours before being fished. At each sample site, habitat and water chemistry measurements were also collected including water temperature, conductivity, dissolved oxygen, and Secchi depth.

COOTES PARADISE EDNA COLLECTION

Water samples were collected at 10 locations throughout Cootes Paradise (Figure 2) on July 2, 2013. Six samples were collected at each site in sterilized 2 L Nalgene bottles and immediately placed in a cooler with ice for transport. Samples were then filtered using vacuum filtration and 1.0 µm pore Whatman™ 47 mm GF/B filters. Filters were packed in sterile containers and placed in a cooler with dry ice. Samples were sent to Trent University for extraction and analysis by staff in the Ontario Ministry of Natural Resources Aquatic Biodiversity and Conservation Unit, following the methodology of Boothroyd (2013).

COOTES PARADISE SPAWNING SURVEY

Fine-mesh fyke nets (1.2 m diameter, 6.35 mm mesh) were set at 14 locations (Figure 3) in shallow waters with emergent vegetation where spawning would potentially occur. Nets were set for approximately 24 hours before being fished. A total of 36 net sets were conducted from June 2 to June 12, 2014. At each sample location, habitat and water chemistry data were also collected. The habitat variables that were measured included water depth, water temperature, conductivity, dissolved oxygen, pH, and turbidity.

RONDEAU BAY TRIBUTARY SPAWNING ASSESSMENT

Fine-mesh fyke nets (1.2 m diameter, 6.35 mm mesh) were set in seven tributary drains that flow into Rondeau Bay. A total of 24 nets were set, from May 21 to 27, 2013. Nets were set in seven tributaries at locations both upstream and downstream of the first major road crossing, as well as at the mouth of each tributary (Figure 4). Nets were set for approximately 24 hours before being fished. At each sample site, habitat data were recorded including water temperature, conductivity, dissolved oxygen, secchi depth, pH, and dominant vegetation. Water samples were also collected from 15 sites (90 total samples) on June 3, 2013 (Figure 5). Water samples were collected in sterilized 2 L Nalgene bottles, then transported, filtered, and analyzed for the presence of Spotted Gar DNA in the same manner as the Cootes Paradise samples.

RONDEAU BAY JUVENILE HABITAT ASSESSMENT

A variety of methods were employed to target juvenile Spotted Gar in Rondeau Bay during the two-week period from July 22, 2013 to Aug 2, 2013 (Figure 6). Quatrefoil light traps were set in shallow waters of Rondeau Bay and its tributaries, for 24 hours at 16 locations. A total of 21 overnight samples were collected. A mamou floating pelagic trawl (2.44 m opening) was used to sample offshore transects of Rondeau Bay. Mamou trawl sampling consisted of 3 contiguous transects, 50 m in length, for a total of 150 m sampled per site. Pelagic trawl samples were collected at 6 sites, for a total of 18 transects sampled. A 10 m bag seine (32 mm mesh) was

deployed in nearshore areas. Sampling was conducted with single replicates at 36 sites throughout Rondeau Bay and its tributaries. At all locations where samples were collected, habitat data were also recorded. Habitat data included water temperature, conductivity, dissolved oxygen, pH, secchi tube depth, turbidity, vegetation cover and substrate type.

An electivity index (Jacobs 1974) was calculated for each habitat variable by comparing the observed habitat where Spotted Gar juveniles were captured to habitat data collected for all sample sites. The electivity index (D) was calculated as follows:

$$D = [r-p] / [(r+p) - 2rp]$$

Where *r* is the proportion of individuals that are found in a particular habitat interval, and *p* is the proportion of the total habitat in that interval (Luttrell et al. 2002). The electivity indices range from -1.00 to +1.00 and are interpreted as follows: -1.00 to -0.50 demonstrates strong avoidance; -0.49 to -0.25 demonstrates mild avoidance; -0.24 to + 0.24 demonstrates no preference or avoidance; +0.25 to +0.49 demonstrates mild preference; and, +0.50 to +1.00 demonstrates strong preference (Moyle and Baltz 1985).

LONG POINT BAY JUVENILE HABITAT ASSESSMENT

A 10 m bag seine (32 mm mesh) was used for targeted sampling of juvenile Spotted Gar in Long Point Bay from July 28 to 31, 2014. Sampling was conducted in shallow, vegetated areas where juvenile Spotted Gar are likely to occur. Three replicate seine hauls of approximately 10 to 15 m in length were conducted at each of 24 sites (Figure 7) for a total of 72 seine hauls. Habitat data including water depth, water temperature, conductivity, dissolved oxygen, pH, Secchi tube depth, turbidity, vegetation cover, and substrate type were recorded at each sample site.

RESULTS

HAMILTON HARBOUR FYKE NETTING

The fyke net sampling conducted in Hamilton Harbour resulted in the capture and identification of 2469 fishes representing 28 species, including three Longnose Gar (Table 1). No Spotted Gar were captured during this sampling event.

COOTES PARADISE EDNA COLLECTION

When the water samples collected from Cootes Paradise were analyzed for the presence of eDNA, a sample from one of the sites (Site 6: 43.27542°N, 79.91564°W) was positive for the presence of Spotted Gar DNA (Figure 2). The mean copy number in this sample was 0.794 copies / µL. The DNA collected was then sequenced and compared to a reference sequence for Spotted Gar. The environmental sample was a complete match to the reference sequence for Spotted Gar Cytochrome oxidase I, confirming the presence of Spotted Gar DNA in the sample. Each of the samples from the other nine sites were negative for the presence of Spotted Gar DNA.

COOTES PARADISE SPAWNING SURVEY

The fyke net sampling in Cootes Paradise resulted in the capture and identification of 474 fishes representing 27 species (Table 1). No Spotted Gar were captured during the sampling of Cootes Paradise.

RONDEAU BAY TRIBUTARY SPAWNING ASSESSMENT

The fyke net sampling in Rondeau Bay and its tributaries during the spawning season of Spotted Gar resulted in the capture of 891 individual fishes representing 28 species (Table 1). A total of 45 Spotted Gar were captured at seven locations (Figure 4). Of the Spotted Gar collected, 37 were captured below the first major road crossing, while eight individuals were captured upstream of the first major road crossing in two different tributaries. Seven individuals were collected in Maclean's Drain above Kent Bridge Road and one individual was collected in Mill Creek above New Scotland Line.

The analysis of the eDNA samples showed positive detections of Spotted Gar DNA at 10 sites (Figure 5), while five sites showed negative detections. The positive detections were found in five tributaries and occurred both upstream and downstream of the first major road crossings (Table 2). Copy number for the positive detections ranged from 4.04 copies / µL to 305.63 copies / µL.

RONDEAU BAY JUVENILE HABITAT ASSESSMENT

The sampling conducted in Rondeau Bay and its tributaries with Quatrefoil light traps resulted in the capture of 91 fishes representing nine different species (Table 1). No Spotted Gar were captured with the Quatrefoil light traps. The sampling with mamou floating trawl resulted in the capture of 3954 fishes representing 17 species (Table 1). No Spotted Gar were captured using the mamou floating trawl. The sampling conducted with 10 m bag seine resulted in the capture of 7306 fishes representing 32 species (Table 1). Eight juvenile Spotted Gar were caught at six different locations, and one adult Spotted Gar was captured. The Spotted Gar juveniles were captured in the nearshore areas of Rondeau Bay, as well as Mill Creek (Figure 6) in mean depth of 0.64 m, mean water temperature of 22.41°C, and mean secchi depth of 0.15 m. The sites had a mix of aquatic vegetation with a mean coverage of the sample area by emergent, floating, and submerged vegetation types of 30%, 9%, and 32%, respectively.

When electivity indices were calculated, Spotted Gar juveniles showed strong avoidance to several habitat intervals including the deepest locations sampled, the highest turbidities and conductivities, and, the areas with pH or dissolved oxygen concentrations at either extreme of the habitats sampled (Table 3). Strong selection was shown for habitats with moderate turbidity levels (50 – 149 NTU). Moderate preference was shown for the shallowest depths (<0.5 m) and temperatures greater than 23.5 °C.

LONG POINT BAY JUVENILE HABITAT ASSESSMENT

The sampling conducted in Long Point Bay resulted in the capture and identification of 2684 fishes representing 26 species (Table 1). No Spotted Gar were captured, however, three juvenile Longnose Gar were collected.

DISCUSSION

HAMILTON HARBOUR AND COOTES PARADISE OCCURRENCE

The physical sampling of Hamilton Harbour provided no evidence of the existence of a Spotted Gar population. Similar results were also found when using eDNA sampling to detect the presence of Spotted Gar in Hamilton Harbour (Boothroyd 2013). The absence of a population of Spotted Gar in Hamilton Harbour is not surprising. In Canada, Spotted Gar prefer warm, shallow, vegetated waters (Glass et al. 2012), such as the habitat found in Rondeau Bay, Lake Erie, where the species is quite numerous (Bouvier and Mandrak 2010). Hamilton Harbour, in contrast to Rondeau Bay, is relatively deep and cool, once supporting a diverse coldwater fish community (Smokorowski et al.1998). Additionally, much of the shallow wetland habitat that was historically present in Hamilton Harbour has been lost due to infilling of nearshore areas (Holmes 1988). Wetland habitat loss, along with agricultural, municipal and industrial pollution, led to a decline in abundance of fishes associated with aquatic vegetation in Hamilton Harbour (Holmes 1988). Temporal data indicate that the fish community in Hamilton Harbour, as measured by Index of Biotic Integrity (IBI), is starting to recover from its most degraded point; however, the community IBI still falls short of recovery targets (Smokorowski et al. 1998; Brousseau and Randall 2008). Given the lack of aquatic vegetation, and the degraded state of the fish community, it is likely that there is no suitable habitat in Hamilton Harbour to sustain a viable population of Spotted Gar. Spotted Gar populations are not historically known from the Lake Ontario watershed, although single individuals have been confirmed (Staton et al. 2012). The limited distribution of Spotted Gar in southwestern Ontario is, in part, due to the timing and route of postglacial dispersal from the Mississipian glacial refugia following the Wisconsinan glacial period (Mandrak and Crossman 1992).

Because the occurrence of a population of Spotted Gar could not be verified in Hamilton Harbour, the source of the individual specimen that was captured in Hamilton Harbour remains uncertain. A possible explanation of its origin is the adjacent Cootes Paradise. Cootes Paradise is a large (250 ha) marsh located at the western end of Hamilton Harbour, formed on a drowned river mouth (Lougheed et al. 1998) and connected to the harbour itself through a narrow channel. Historically, the Cootes Paradise marsh had up to 90% macrophyte coverage, although it has experienced habitat degradation due to pollution and disturbance by invasive species, resulting in a decline in vegetation coverage to 15% of the total area by the 1990s (Lougheed et al. 1998). Subsequent to the adoption of the Hamilton Harbour Remedial Action Plan, a fishway was constructed to exclude Common Carp (*Cyprinus carpio*) from Cootes Paradise in 1997. The exclusion of Common Carp, along with other restoration efforts, has led to an improvement in water quality and an increase in the number of piscivorous fishes present in the Cootes Paradise marsh, although high turbidity and nutrient concentrations remain a barrier to expansion of macrophyte coverage (Thomasen and Chow-Fraser 2012).

Despite the degraded state of the Cootes Paradise marsh, there is still a large area of aquatic macrophyte growth (Lougheed et al. 1998), providing potential habitat for a population of Spotted Gar. Additionally, the fishway is designed to impede passage of larger fishes, such as adult Common Carp, but a slim-bodied fish, such as a juvenile Spotted Gar, could pass through undetected. Given that the Cootes Paradise marsh contains more appropriate habitat for Spotted Gar than Hamilton Harbour itself, Cootes Paradise may be the source of the Hamilton Harbour specimen. Although no Spotted Gar have been recorded from Cootes Paradise

(Staton et al. 2012), the positive eDNA detection in this study raises the possibility of a previously unknown population of Spotted Gar being present in the marsh. The use of eDNA has been successful in detecting the presence of rare aquatic species (Goldberg et al. 2011; Jerde et al. 2012), although the possibility that the source of the eDNA is not a live individual (ECALS 2013) necessitates traditional sampling to verify the existence of a population.

To verify the presence of Spotted Gar in the system, targeted sampling with fine-mesh fyke nets was conducted in the Cootes Paradise marsh during the spring spawning season in 2014. Sampling was conducted in the vicinity of the mouth of Spencer Creek, the largest area of emergent vegetation in Cootes Paradise. The area that was sampled was also the location of the positive eDNA detection in the previous summer. This sampling resulted in no captures of Spotted Gar, thus, the presence of a population of the species residing in Cootes Paradise is not confirmed.

RONDEAU BAY SPAWNING HABITAT

The results of the spawning survey in the Rondeau Bay tributaries clearly demonstrate that Spotted Gar utilize the tributaries during the spawning season. Presently, the designation of critical habitat for the species in Rondeau Bay includes the bay itself, and the mouths of tributaries upstream to where a defined channel begins (Staton et al. 2012), downstream of the first major road crossing. Positive detections of Spotted Gar, through traditional netting methods, as well as eDNA sampling, indicate that the species is utilizing areas upstream of the designated critical habitat, likely for spawning purposes. Spawning has been previously documented in the bay (Glass et al. 2012), but not in the tributaries, which are agricultural drains and potentially exposed to agricultural drain maintenance. Maintenance of these drains would remove aquatic vegetation (Stammler et al. 2008) utilized by Spotted Gar. Spotted Gar were observed (through eDNA and traditional sampling methods) well above the currently designated critical habitat and likely travel upstream as far as the first permanent barrier to fish passage to spawn amongst emergent vegetation.

There were five sites where Spotted Gar was detected using eDNA techniques but not with traditional methods, while Spotted Gar were captured at an additional three sites that did not show positive detections through eDNA sampling (Table 2). One of the possible explanations for this finding is the time lag between sampling with the two techniques. The traditional sampling was conducted several days in advance of the eDNA sampling. If Spotted Gar were in the area during traditional sampling, and subsequently moved out of the area during the period that eDNA samples were collected, this would account for the three sites that tested negative using eDNA techniques despite captures with traditional sampling. Conversely, if Spotted Gar were not present during traditional sampling and moved in afterward, this could account for positive eDNA detections despite not capturing the species with traditional techniques at a given site. Additionally, detection of species at risk with traditional sampling techniques is typically imperfect (Dextrase et al. 2014), thus, if a Spotted Gar were present in low numbers at a site eDNA techniques may show positive detection despite not capturing the species through traditional means. This would reinforce the utility of eDNA as a sampling method for rare species. Time lag between sampling events and imperfect detection, however, does not account for all observed discrepancies. While collecting eDNA samples, Spotted Gar were visually identified in the vicinity of four eDNA sample sites. Positive detections resulted from samples at three of these sites (220513-001, 220513-003, 220513-004), while one of the sites

(220513-002) where Spotted Gar were visually evident tested negative for the presence of Spotted Gar using eDNA techniques. Additionally, positive eDNA detections of Spotted Gar in the Thames River / Jeanette's Creek (Boothroyd 2013) have not been verified through traditional means, despite intensive sampling of the area by DFO field crews, including the collection of over 400 Longnose Gar (*L. osseus*) (DFO, unpubl. data). The discrepancies between eDNA sampling and traditional sampling methods in this study (Rondeau Bay, Cootes Paradise) and the Thames River / Jeanette's Creek study indicate that additional research is required to verify the specificity of the primers used for identification of Spotted Gar eDNA. Research to quantify detection probability using eDNA methods in a variety of environmental conditions is also recommended.

RONDEAU BAY JUVENILE HABITAT

The study of habitat use by juvenile Spotted Gar indicated that the presence of aquatic vegetation is important for early life stages. Tracking of adult Spotted Gar has shown that mixed macrophyte beds are an important habitat feature for the species (Glass et al. 2012), and the density of the cover is influential on the species' feeding success (Ostrand et al. 2004). Juvenile Spotted Gar likely use the aquatic vegetation for cover to escape predators, as well as places to ambush prey.

Electivity indices, based on the capture locations of Spotted Gar juveniles, differ from neutral selection for several habitat intervals, indicating that Spotted Gar juveniles are not randomly distributed throughout the study area. Electivity indices show that juvenile Spotted Gar prefer shallow depths (<0.5 m) and, similarly, adults showed strong selection for depths less than 0.5 m during the summer months (Glass et al. 2012). Temperature preference for juveniles was also similar to that exhibited by adults (Glass et al. 2012), where the warmest temperatures were preferred. The preferred temperature for juvenile Spotted Gar, like that of the adults, was higher than the preferred temperature listed for the species by Coker et al. (2001). There was no similarity in the preference for specific pH or conductivity between juveniles and adults. In both cases, the range of observations varied greatly between the two study periods.

Moderate turbidity levels (50 – 150 ntu) were preferred by juvenile Spotted Gar, while the most turbid waters were strongly avoided. Avoidance of the most turbid waters is expected since this species is a visual predator (Scott and Crossman 1998). The preference for moderately turbid water is surprising though, since turbidity is cited as a threat to the species in Canada (Staton et al. 2012) and even low levels of turbidity have been shown to affect hatching success of Spotted Gar in laboratory studies (Gray et al. 2012). The preference of moderate turbidity levels may increase the feeding success of Spotted Gar juveniles as turbidity has been shown to lessen the ability of prey fish to recognize predators (Ferrari et al. 2010), and lessen the effectiveness of anti-predator behavior (Abrahams and Kattenfeld 1997).

The conclusions about habitat use by juvenile Spotted Gar should be considered preliminary, as the sample size of field observations is small; however, this study includes the only observations to date of Spotted Gar juveniles in the wild in Canada. The juvenile life stage remains highly understudied. There is a dearth of literature regarding the juvenile life stage for this species, not just in Canada, but throughout the species' range. Because the life stage is understudied, and since mortality of the juvenile life stage is predicted to have the most impact on the recovery of

the species in Canada (Young and Koops 2010), it is important to conduct further research on the distribution and habitat use of juvenile Spotted Gar in Canada.

Future efforts to collect Spotted Gar juveniles should employ seine netting. This technique proved to be the most successful of the sampling methods. Quatrefoil light traps were used in many of the same locations where juveniles were later captured with the seine, thus, light traps were ineffective. The effectiveness of the Quatrefoil light traps may have been diminished due to the size of juvenile Spotted Gar during the sampling period being too large to recruit into the trap. Additionally, the moon phase at the time of sampling (full moon) may have reduced the attractiveness of the light emitted by the traps. The mamou floating trawl was also not successful in capturing juvenile Spotted Gar, although it is unclear whether this was due to the gear being deployed in deeper waters where juveniles were not found, or a deficiency with the gear itself.

LONG POINT BAY JUVENILE HABITAT

The sampling of Long Point Bay conducted in the summer of 2014 resulted in no captures of Spotted Gar juveniles, despite employing the technique that was proven effective in Rondeau Bay (10 m bag seine), and concentrating effort in locations where adult Spotted Gar were captured in the past. The sampling effort in Long Point Bay was also greater than the seining effort in Rondeau Bay that resulted in the capture of eight juveniles. The Long Point Bay population is the smallest of the Spotted Gar populations in Canada (Staton et al. 2012), with only 17 individuals recorded from the bay since 1947. The lack of juvenile captures, despite significant sampling effort, points to low levels of successful reproduction in Long Point Bay. The low level of successful reproduction, combined with recent genetic evidence that Long Point is a population sink (Glass et al. in prep.), suggests that this population may not be viable in the long term.

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Table 1. Fish species and number of individuals collected at Hamilton Harbour, Rondeau Bay, Cootes Paradise, and Long Point Bay using traditional gear types.

Species	Hamilton Harbour Fyke Net	Rondeau Fyke Net	Rondeau Quatrefoil Light Trap	Rondeau Seine	Rondeau Floating Trawl	Cootes Paradise Fyke Net	Long Point Bay Seine
Petromyzon marinus	-	-	-	-	-	1	-
Lepisosteus oculatus	-	45	-	9	-	-	-
Lepisosteus osseus	3	3	-	-	-	-	3
Amia calva	4	9	-	3	-	8	-
Alosa pseudoharengus	11	-	-	-	-	-	-
Dorosoma cepedianum	1	-	4	3215	-	-	13
Cyprinidae	-	-	1	-	-	-	-
Carassius auratus	1	-	-	43	4	9	6
Cyprinus carpio	5	5	-	16	-	3	-
Cyprinella spiloptera	-	-	-	-	-	-	2
Luxilus cornutus	-	-	-	1	-	2	-
Notemigonus crysoleucas	10	68	1	263	5	1	12
Notropis anogenus	-	-	-	-	-	-	20
Notropis atherinoides	49	19	-	16	-	-	18
Notropis heterodon	-	17	-	25	8	-	174
Notropis heterolepis	-	-	-	-	-	-	16
Notropis hudsonius	145	103	-	-	25	396	5
Notropis volucellus	-	-	-	3	-	-	3
Pimephales notatus	32	223	-	214	35	6	72
Pimephales promelas	-	2	-	1	-	6	-
Scardinius erythrophthalmus	-	-	-	-	-	12	-
Nocomis biguttatus	-	-	-	-	-	1	-
Semotilus atromaculatus	-	-	-	-	-	2	-
Catostomus commersonii	5	2	-	2	-	8	-
Ictiobus spp.	-	-	-	19	-	-	-
Ameiurus melas	3	2	-	-	-	-	-
Ameiurus natalis	-	51	-	3	1	-	-
Ameiurus nebulosus	27	34	-	22	-	50	17

Species	Hamilton Harbour Fyke Net	Rondeau Fyke Net	Rondeau Quatrefoil Light Trap	Rondeau Seine	Rondeau Floating Trawl	Cootes Paradise Fyke Net	Long Point Bay Seine
Ictalurus punctatus	1	-	-	3	-	-	-
Noturus gyrinus	-	5	-	1	-	1	1
Osmerus mordax	4	-	-	-	-	-	-
Salmonidae	4	-	-	-	-	-	-
Oncorhynchus mykiss	-	-	-	-	-	7	-
Salmo trutta	-	-	-	-	-	1	-
Salvelinus namaycush	1	-	-	-	-	-	-
Esox lucius	4	9	-	2	2	20	1
Umbra limi	-	2	-	1	-	-	-
Labidesthes sicculus	5	1	8	173	9	-	694
Fundulus diaphanus	-	18	-	142	11	-	19
Culaea inconstans	-	-	-	-	-	1	-
Morone americana	660	1	-	-	-	22	-
Centrarchidae	-	-	2	-	-	-	-
Ambloplites rupestris	31	19	-	7	7	15	118
Lepomis spp.	-	-	2	2	1345	-	365
Lepomis cyanellus	2	1	22	3	-	2	-
Lepomis gibbosus	600	31	8	103	107	62	186
Lepomis gulosus	-	1	-	-	-	-	-
Lepomis macrochirus	525	96	29	2654	2153	36	216
Micropterus salmoides	21	4	11	245	134	1	664
Pomoxis annularis	-	-	1	9	-	-	-
Pomoxis nigromaculatus	1	8	1	82	91	-	1
Etheostoma exile	-	-	-	1	1	-	-
Etheostoma nigrum	-	-	-	11	-	-	2
Perca flavescens	13	7	-	12	14	33	7
Percina caprodes	2	-	-	-	-	3	2
Neogobius melanostomus	300	-	-	10	1	6	47

Table 2. Detection of Spotted Gar at Rondeau Bay sample locations using eDNA and traditional gear types during spawning survey. 0 indicates no detection; 1 indicates positive detection.

Site Number	eDNA Detection	Traditional Detection
210513-001	1	1
210513-002	1	0
210513-003	1	0
210513-004	0	1
210513-005	0	0
220513-001	1	NA
220513-002	0	1
220513-003	1	1
220513-004	1	1
220513-005	1	0
220513-006	1	1
230513-001	0	1
230513-002	1	0
230513-003	0	0
270513-001	NA	0
270513-002	1	0
270513-003	NA	0
270513-004	NA	0

Table 3. Electivity indices and selection level for habitat variable intervals used by juvenile Spotted Gar in Rondeau Bay

Habitat Variable	Habitat Interval	Electivity Index	Selection Level
Depth (m)	<0.5	0.33	Moderate selection
	0.5 – 0.749	0.10	Neutral selection
	0.75 – 1.0	0.03	Neutral selection
	>1.0	-1	Strong avoidance
Temperature (˚C)	<19.5	-0.19	Neutral selection
	19.5 – 21.49	-0.23	Moderate avoidance
	21.5 – 23.49	0.03	Neutral selection
	≥23.5	0.26	Moderate selection
Turbidity (ntu)	<50	-0.19	Neutral selection
	50 – 149.9	0.74	Strong selection
	150 – 249.9	-0.19	Neutral selection
	≥250	-1	Strong avoidance
Conductivity (µs)	<400	0.24	Neutral selection
	400 – 599.9	-0.23	Neutral selection
	600 – 799.9	0.35	Moderate selection
	≥800	-1	Strong avoidance
Dissolved Oxygen (mg/L)	<5.0	-1	Strong avoidance
	5.0 – 9.99	0.24	Neutral selection
	10.0 – 14.99	-0.72	Strong avoidance
	≥15.0	-0.72	Strong avoidance
pH	<7.66	-1	Strong avoidance
	7.67 – 8.32	-0.10	Neutral selection
	8.33 – 8.99	-0.72	Strong avoidance
	≥9.0	-0.42	Moderate avoidance

Figure 1. Sample locations in Hamilton Harbour. Fishes were collected using fine-mesh fyke nets set for 24 hours.

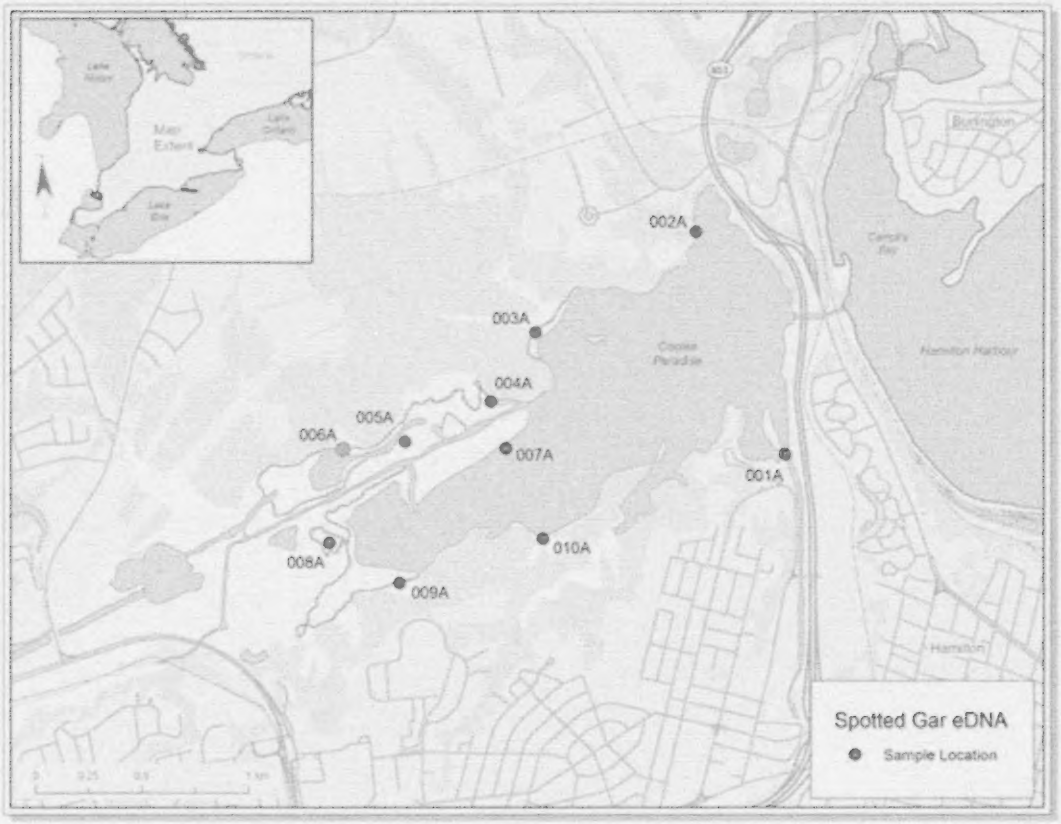

Figure 2. Cootes Paradise Marsh eDNA water sample collection locations. Green circle indicates sample location that tested positive for the presence of Spotted Gar DNA.

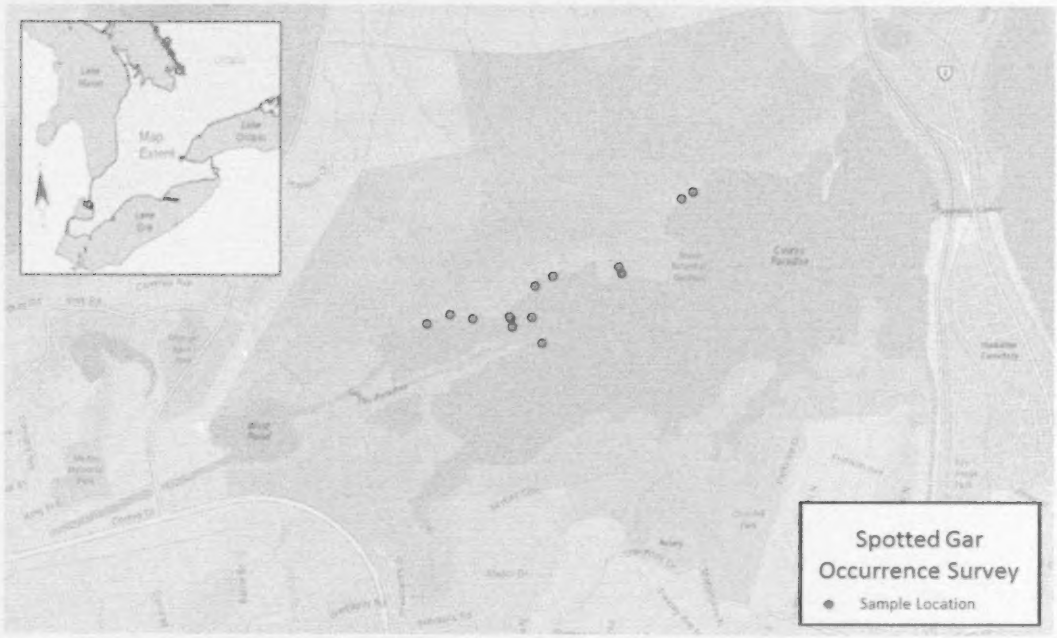

Figure 3. Fish sample collection locations in Cootes Paradise Marsh. Fishes were collected with fine-mesh fyke nets set for 24 hours.

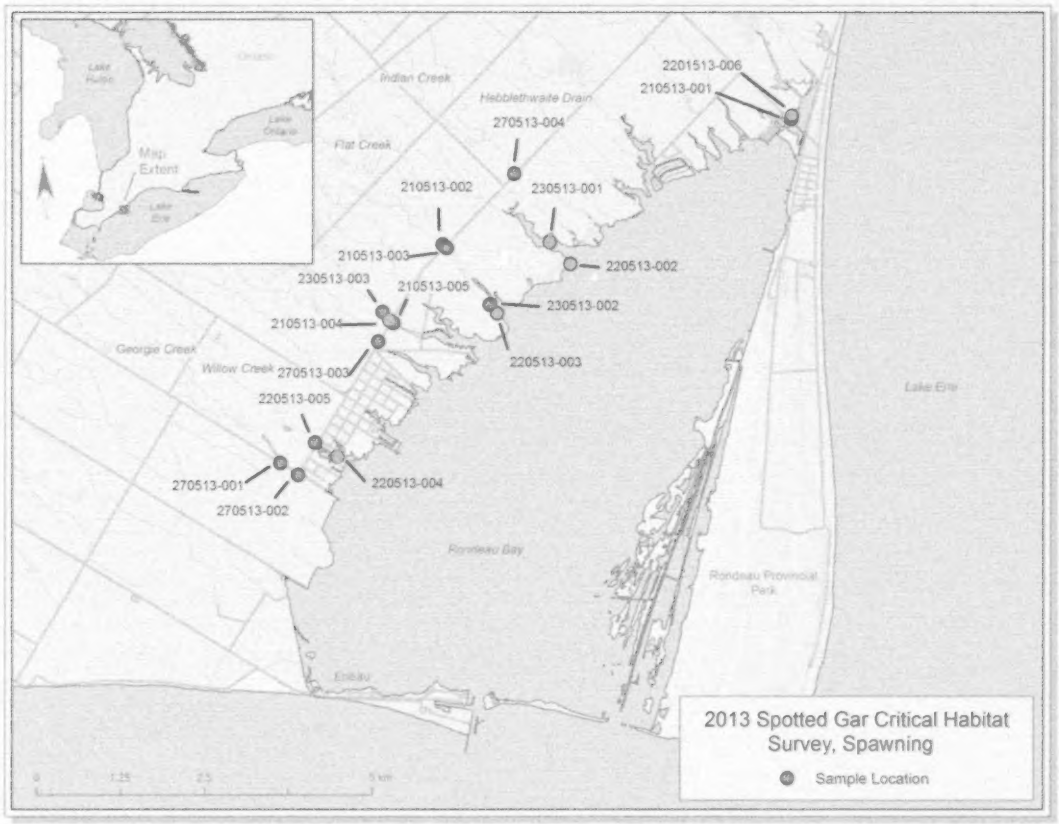

Figure 4. Sample locations in Rondeau Bay and surrounding tributaries. Fishes were collected using fine-mesh fyke nets. Green circles represent locations where Spotted Gar were captured.

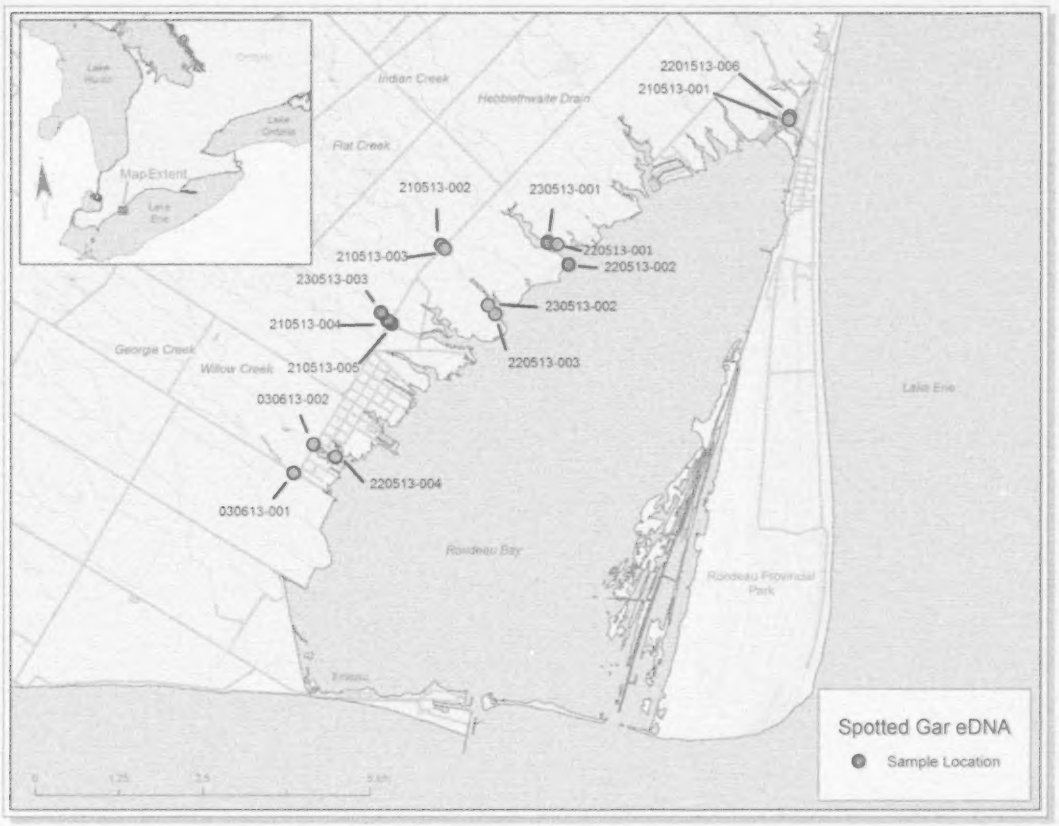

Figure 5. Environmental DNA water sample collection sites in Rondeau Bay and surrounding tributaries. Green circles indicate samples that tested positive for the presence of Spotted Gar eDNA.

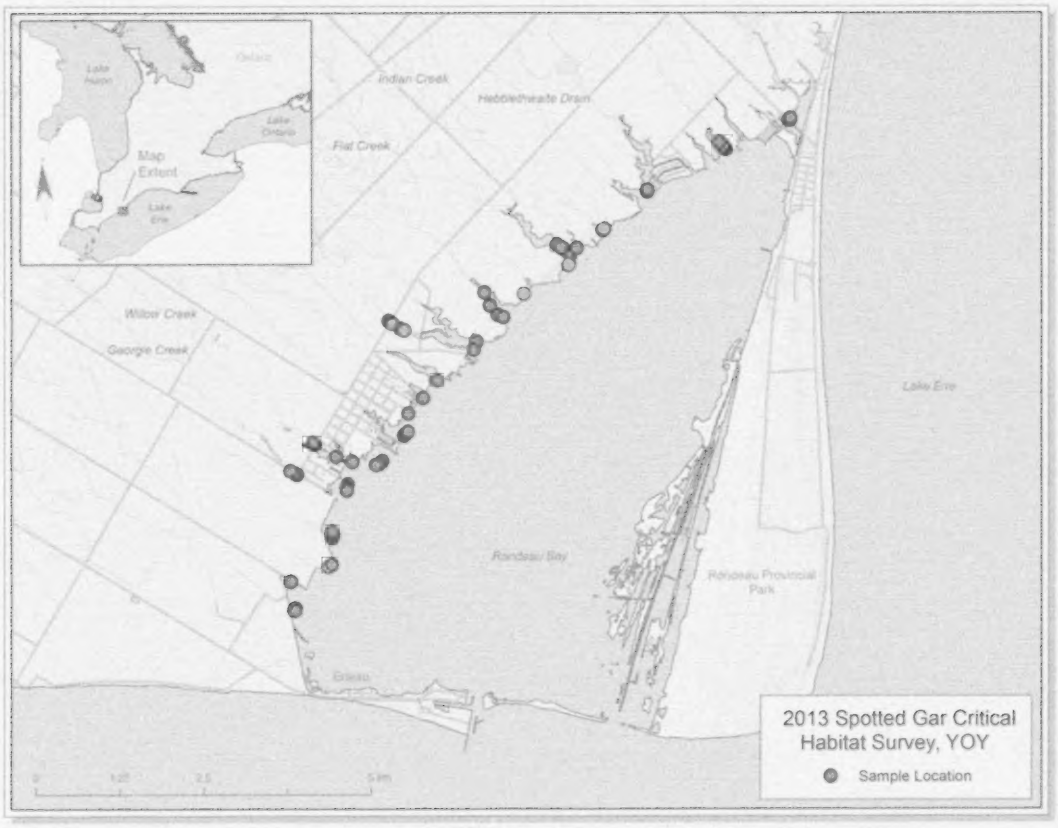

Figure 6. Spotted Gar juvenile habitat survey sample locations in Rondeau Bay and surrounding tributaries. Green circles indicate locations where juvenile Spotted Gar were successfully collected.

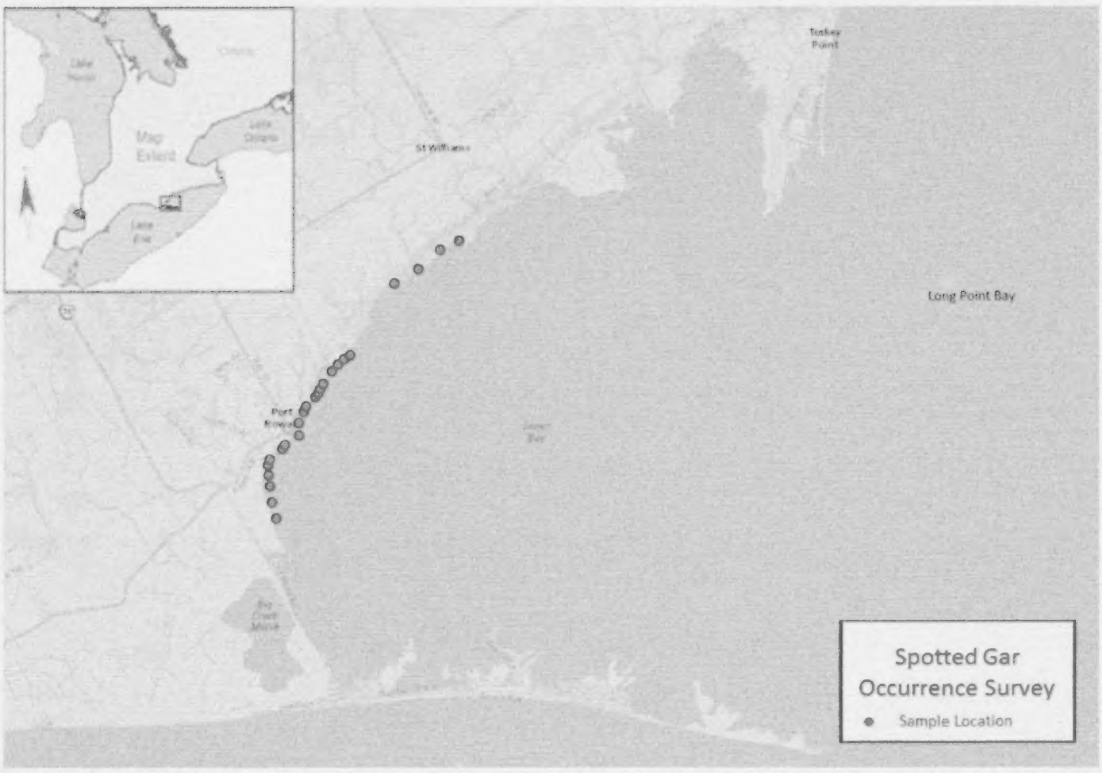

Figure 7. Fish sample collection locations in Long Point Bay. Fishes were collected with a 10 m bag seine.